ON THE STRUCTURE OF SOME MICROSPORIDIAN SPORES *

R. KUDO

The structure of the spores of Microsporidia has been variously described by several authors for different species. Even in one and the same species, the observations of many investigators do not seem to agree. This controversy may be attributed partly to the minuteness of the object and partly to the difference in the technic used. On examining the numerous papers on Microsporidia, one would be impressed by the fact that the majority of the authors do not state their observations with positiveness.

A more or less generally accepted conception of the structure of the Microsporidian spore seems to have been given by Mercier (1908) for *Thelohania giardi* (length 5 to 6μ, after Thélohan). Mercier observed that the spore is covered with a bivalve shell, each valve developing from an uninucleated parietal cell, that the spirally coiled polar filament is contained in a polar capsule with a nucleus, that the girdle-shaped sporoplasm with at first two, later four nuclei, surrounds the polar capsule, and that a vacuole is present at each pole of the spore. This view, on the whole, has been confirmed by Schröder (1908), Stempell (1909), Fantham and Porter (1912, 1914), Strickland (1913), Kudo (1916), and others, altho their observations differ in details.

On the other hand, Schuberg (1910) noticed in the spores of *Plistophora longifilis* (macrospore, 12μ long, 6μ wide; microspore, 3μ long, 2μ wide) that the girdle-shaped sporoplasm which is circular in cross-section, contains a single nucleus, that the polar filament is coiled directly under the shell mostly in the posterior portion of the intrasporal space, that the so-called polar capsule does not occur in the Microsporidian spore, and that the nuclei observed by other authors, are none others than the metachromatic granules. The same view has been maintained by Omori (1912), Weissenberg (1911, 1913) and Debaisieux (1913, 1915).

Léger and Hesse (1916) described an interesting type of Microsporidia under the generic name of Mrazekia. The spores are of cylindrical or tubular form, and show an entirely different structure compared with other genera. The polar filament is differentiated into two parts. No polar capsule is mentioned as present, the polar fila-

* Contributions from the Zoological Laboratory of the University of Illinois, No. 151.

ment being coiled directly inside of the shell. Instead of being in form like a girdle, the binucleated sporoplasm is a rounded and more or less well defined body embedded in a clear space at the posterior portion of the spore.

The same authors (1916a) later reported a similar observation made on the structure of the spore of *Plistophora macrospora* (8.5μ long, 4.24μ wide, after Cépède). They mentioned that the polar capsule lies closely to the shell, occupying the greater part of the intrasporal space, that the polar filament is coiled in the capsule without a central axis, that the sporoplasm is a rounded binucleated body embedded in the posterior vacuole of the spore, that the girdle-shaped structure which was thought to be the sporoplasm by numerous authors is none other than the retracted substance composing the polar capsule so that one or two turns of the polar filament were mistaken in optical cross-section as a variable number of nuclei, and that the granule in the posterior vacuole which was designated as a metachromatic granule by some authors, is none other than the nucleus of the true sporoplasm. Georgévitch (1917) agreed with the above mentioned view in his study on *Mariona marionis,* altho he noticed that the polar capsule was entirely absent in some spores.

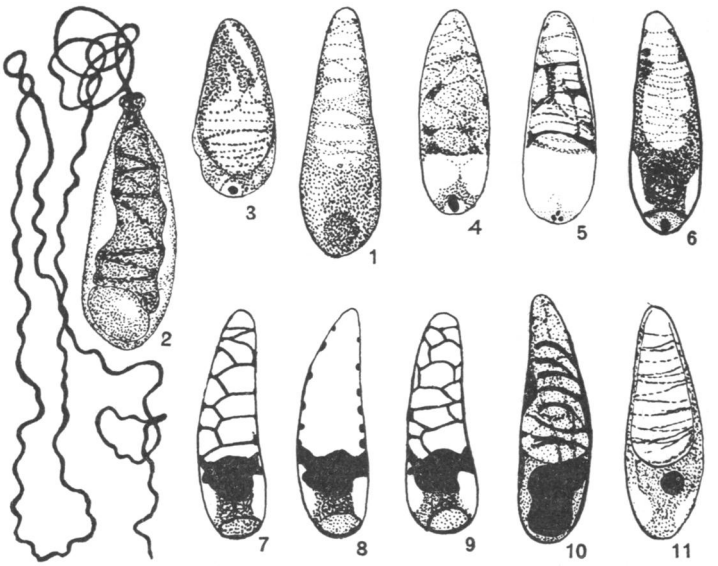
The writer has recently obtained four new forms of Microsporidia from the vicinity of Urbana, Illinois. As their occurrence is rather rare and their life history is now being studied by both natural and artificial infections, it will take some time to complete the work.

One of the parasites, which is a rare parasite in the larvae of *Culex pipiens* from a limited water area in Urbana, proved to be well fitted for the study on the structure of the spore. The spore is from 12 to 13.5μ in length, and 4μ in breadth as measured in stained materials. It is, therefore, one of the largest microsporidian spores that have been recorded. Altho some of the spores of *Plistophora longifilis* and *Thelohania legeri* have the same length, the majority of the spores of these two species are small, while the present form has its advantage in having spores of uniformly large dimensions. Besides, the shell is very thin so that the internal structure could, to some extent, be made out in vivo.

The spore is elongated pyriform usually slightly bent toward one side. It is circular in cross-section. The posterior end is broadly rounded, while the anterior extremity is less rounded, tho not attenuated.

In the fresh state, the spore exhibits marked vacuolation thruout the intrasporal space. For about two-thirds of the anterior portion, a fine polar filament coiled like a network can be seen (Fig. 1), which becomes more distinctly visible when stained, while the posterior one-third is occupied by a finely granulated protoplasmic mass which often

contains a refringent body near its extremity. When fresh spores are subjected to mechanical pressure (Kudo, 1913), and stained by Fontana's method, the extruded polar filament is distinctly recognizable (Fig. 2). This filament is uniformly thick, shows usually a wavy course, and reaches a length of 230μ. The writer does not think this as an average length but records it here as the longest one found so far. In Figure 2 is shown not only the extruded polar filament, but also its remaining part coiled spirally inside of the capsule. The same figure gives at the same time strong evidence for the presence of a particular polar capsule with its polar filament. The shell does not



Spores of *Thelohania magna* nov. spec. × 2360. Fig. 1. A fresh spore. Fig. 2. A spore mechanically pressed, and stained after Fontana. Fig. 3. A young spore stained with Giemsa's stain. Figs. 4-6. Spores stained with Giemsa's stain. Figs. 7-9. Three different views of a single spore stained with Giemsa's stain. Fig. 7. The lower surface view. Fig. 8. The optical section. Fig. 9. The upper surface view. Fig. 10. A spore somewhat deeply stained with Giemsa's stain. Fig. 11. A spore stained with Delafield's hematoxylin.

exhibit any sutural line that might suggest a bivalve nature such as one sees in a Myxosporidian spore either in fresh or stained preparations.

When fixed with Schaudinn's fluid, and stained with Giemsa's stain followed by acetone dehydration, Heidenhain's iron hematoxylin, or Delafield's hematoxylin, the spore shows its various structures very distinctly. Inside of the shell, a large pyriform polar capsule becomes more visible together with the polar filament. The polar capsule, 7.5μ in length, occupies about two-thirds of the anterior portion of the

spore as studied in the fresh state. The foramen of the capsule can not be seen clearly, but the fact that the polar capsule opens at the anterior tip of the spore is distinctly shown in Figure 2. The wall of the polar capsule is comparatively thin, and is very faintly stained in many spores treated with Giemsa's stain (Figs. 4, 5, 7-9). In spores stained deeply with the same stain, however, the polar capsule is recognizable as a reddish colored sack (Fig. 10). In younger spores it is well seen (Fig. 3). It is distinctly recognizable when the spore is brought under the influence of mechanical pressure (Fig. 2). A polar capsule of similar appearance was observed by Schrröder (1914) in *Thelohania acuta,* altho the same author did not trace out the filament. The polar filament is coiled spirally along the inner surface of the polar capsule. Its spiral course begins at the anterior tip of the capsule, and does not differentiate a central axis, altho some longitudinal courses were often seen in the posterior portion of the capsule (Fig. 10). Figure 3 shows the developing polar filament in a young spore; the windings are more or less clearly visible. In a deeply stained spore, the spiral can be recognized distinctly (Fig. 10). Three different views of a single spore treated with Giemsa's stain are shown in Figures 7 to 9, which exhibit the spiral course more distinctly along the inner surface of the polar capsule than any other spores. The spirality of the present form is, therefore, somewhat similar to that of *Plistophora macrospora* (Léger and Hesse, 1916a), of *Nosema bombycis* (Kudo, 1916), and of most of the Myxosporidian spores (Auerbach, Kudo, Davis, etc.); but differs from Stempell's (1909) observations on *Nosema bombycis* and from Mrazekia studied by Léger and Hesse (1916).

The rounded sporoplasm occupies the posterior third of the spore. In fixed preparations a clear space is seen on its lateral side (Figs. 6, 7-9, 11). The nucleus is a comparatively large rounded compact mass embedded in the sporoplasm, and shows typical nuclear staining by the above mentioned stains. It is well differentiated in spores stained with Delafield's hematoxylin (Fig. 11). In every spore stained less deeply with Giemsa's stain the nucleus is represented by a single, or two or three smaller chromatic granules situated regularly at the posterior tip of the spore (Figs. 3-6). No nucleus for the polar capsule or the shell has been recognized. Schuberg (1910) noticed a similar fact in *Plistophora longifilis* as was stated before.

The other three forms have spores of much smaller dimensions, and so far have not shown any fact regarding their structure other than the observations which were presented by the present writer in his paper on *Nosema bombycis* (Kudo, 1916).

SUMMARY

The spore of *Thelohania magna* nov. spec. is of exceptionally large dimensions. Microsporidian spores are not so similarly built as those of different genera of Myxosporidia. A diversity in the structure of Microsporidian spores is recognized with at least two categories; one type, *Nosema bombycis* and the other type, *Thelohania magna*. The latter has a distinct polar capsule with spirally coiled polar filament without central axis; it has a rounded sporoplasm containing a single nucleus. Combination of mechanical pressure and Fontana's staining is especially favorable for the study of the extrudèd polar filament, and also some structures in the spores. To this type may belong *Thelohania acuta, Plistophora elegans* and *P. macrospora*.

It is interesting to note that altho the parasite attacks only the adipose tissue of the host, infected larvae die more rapidly in captivity than normal ones. So far pupae and adults have been found to be free from infection, which suggests a fatal effect of the parasite upon the host body.

WORKS CITED

Kudo, R. 1916.—Contributions to the Study of Parasitic Protozoa. I. On the Structure and Life History of *Nosema bombycis* Nägeli. Bull. Imp. Seric. Exp. Stat., Tokio, 1: 31-51.

Léger, L., and Ed. Hesse. 1916.—Mrazekia, genre nouveau de Microsporidies à spores tubuleuses. C. R. soc. biol., 79: 345-348.

1916a.—Sur la structure de la spore des Microsporidies. C. R. soc. biol., 79: 1049-1053.

Mercier, L. 1908.—Sur le development et la structure des spores de *Thelohania giardi* Henneguy. C. R. acad. sci., 146: 34-38.

Schuberg, A. 1910.—Ueber Mikrosporidien aus dem Hoden der Barbe und durch sie verursachte Hypertrophie der Kerne. Arb. kais. Gesundh., 33: 401-434.